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CARTILAGE OF THE INTERVERTEBRAL DISC END-PLATE
A HISTOLOGICAL, HISTOCHEMICAL, AND FINE STRUCTURE STUDY

NOEL S. NUSSBAUM

AUGUST 1981

AEROSPACE MEDICAL RESEARCH LABORATORY
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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Henning E. von Gierke

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The cartilaginous end plates define the anatomical boundaries of the disc, serve as an area of contact with the vertebral body, and act as a semi-peremeable membrane. The anatomic relationships that exist between the end plate and immediately adjacent structures can be better understood if ultrastructure is interpreted in conjunction with established histology.
Undecalcified sections of Rhesus (Macaca mulatta) spinal segments were prepared, for both light and scanning electron microscopy, from 17 adolescent and young

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adults with radiographically normal spines. Alternate thick (100 μm) and thin (12 μm) sections were cut at 0.5 mm intervals through quick-frozen tissues, embedded in sodium carboxymethyl cellulose, on a LKB 2250 PMV Cryomicrotome. Thin sections were stained for histological and histochemical examination and thick sections were examined with an Etec Auto Scan electron microscope.
End plate cartilage from mature spines demonstrate a micro-heterogeneous structure. Strut-like inter-territorial regions (ca. 50 μm wide), impregnated with calcium salts (von Kossa), separate and define inter-connecting cellular and pericellular domains. The proteoglycan content of the pericellular matrix stains more intensely (Safranin O) then does the inter-territorial region. Structural and histochemical data suggest the presence of a cell-mediated diffusion pathway allowing for trans-endplate movement of disc nutrients. This interpretation contrasts with the classical view of the end plate as a passive diffusion pathway but may explain the observation that the bone-disc interface is only partially permeable in contrast to the freely permeable peripheral annulus.
Degenerative alterations in this system could be expected to compromise disc function. A better understanding of these relationships is vital for the development of treatment strategies designed to alleviate and/or correct the effects of acute and chronic disc degeneration.

PREFACE

This work was performed while the author was a Visiting Professor at the Biodynamic Effects Branch (BBD),Biodynamics and Bioengineering Division, Air Force Aerospace Medical Research Laboratory. The assistance of K. C. Smith (BBD), R. Nieser and D. Mattie (Toxic Hazards Division, Pathology Branch) is appreciated.

TABLE OF CONTENTS

	Page
INTRODUCTION	3
OBJECTIVE	3
MATERIALS AND METHODS	4
RESULTS	5
DISCUSSION	12
REFERENCE	13

LIST OF ILLUSTRATIONS

Figure

1 View of microtome within LKB Cryochest. Specimen block is to left of knifeholder. Frontal sections are being cut. 1/6X (Total magnification given for all figures) 6

2 Example of a reference photograph taken every 0.5 mm as specimen is sectioned. In this case the animal is identified as rhesus 495 and the section is 4.5 mm into the vertebral centrum in the sagital plane. 3/4X 6

3 Sagital section of rhesus T^2-T^3 Intervertebral Disc. Trabecular bone of vertebral body encloses marrow spaces. Cartilagenous end-plates are separated by growth cartilage from the adjacent spongy bone. Mallory, 7.5X 7

4 Frontal Section of rhesus T^6-T^7 Disc. Vascularization of inferior end-plate is continuous but is restricted to lateral ends of superior. Mallory, 7.5X 7

5 Low power photomicrograph of anterior margin of inferior end-plate. Vascular buds are surrounded by ossifying matrix. Collagen bundles of annulus fibrosus above terminate in cartilage of end-plate. Light staining growth cartilage is located between end-plate and subchondral bone of vertebral centrum below. 50X 8

6 Higher power view of disc-bone junction from young animal. Note absence of vascular buds and ossification centers in end-plate cartilage. Other features the same as fig. 5. 100X 8

7 Disc-bone junction below nucleus pulposus. Termination of annular collagen bundles associated with narrow zone of end-plate cartilage.Cartilage exhibits mosaic of staining intensities with darker territorial regions surrounding cells and interterritorial zone appearing lighter. Mallory stain. 150X 9

8 Higher power view of end-plate cartilage demonstrating mosaic of aniline blue staining associated with territorial (T) and interterritorial (I) regions. Mallory stain 450X 9

9 Positive histochemical reaction for proteoglycans in territorial zone. Alcian blue stain. 900X 10

10 Left. Positive von Kossa reaction demonstrating calcium salts in interterritorial zone. Right. Positive alcian blue reaction for territorial regions. 200X 10

11 Scanning electron micrograph (SEM) of end-plate cartilage. Cellular territorial (T) domains appear depressed between interterritorial (I) calcified regions. Compare with fig. 9. 410X 11

12 SEM of cellular elements, territorial zone and adjacent calcified interterritorial matrix. 1400X 11

INTRODUCTION

Age-dependent fibrotic degeneration of the intervertebral disc seriously compromises energy dissipation within the compression loaded spine. The loss of viscoelasticity associated with fibrosis of the nucleus pulposus has been identified with impaired nutritive flow from adjacent subchondral bone. Development of a dynamic model of the human spine is in turn dependent upon quantification of relevant parameters. The influence of age-driven variables affecting these parameters must also be estimated.

OBJECTIVE

The objectives of this study are twofold. The first is to describe the histological, histochemical, and ultrastructural features of the cartilagenous end plates of the intervertebral discs of the rhesus monkey (Macaca mulatta) as derived from examination of undecalcified sections. The second is to relate this structural information to current theories of disc nutrition, and age-dependent fibrotic degeneration.

MATERIALS AND METHOD

Vertebral segments comprising the T^7 - T^{11} region were isolated from fresh quick-frozen spines of female rhesus monkeys ranging in weight from 12.5 to 21.5 pounds. Five spines from fresh material and an additional 12 long-term frozen spines were examined. Frontal, sagital and horizontal sections were cut with the LKB 2250 PMV cryomicrotome. Frozen, undecalcified specimens were embedded in carboxymethyl cellulose within a frame, and frozen in hexane cooled with dry ice (-75°C). The frozen block was then sectioned at 0.5 mm intervals (Fig. 1). Photographs of the block face (Fig. 2), a series of thin sections at 10-12 micrometers, and a thick section at 100 micrometers were collected at each 0.5 mm interval.

Sections were collected on transparent tape. Thin sections were fixed immediately in:

95% Ethyl alcohol ..... 70 ml
10% Formalin ..... 29 ml
Acetic acid (glacial) ..... 1 ml

Fixation for 2 minutes was foilowed by a distilled water rinse and then the sections were allowed to air dry completely before staining. Adjacent sections were stained with H-E, Mallory's connective tissue stain, von Kossa for calcium salts, and Alcian blue-light green for proteoglycans (Humason, 1972). Microphotographs were prepared with a Zeiss Photomicroscope.

Thick sections for scanning electron microscopy were dehydrated overnight within the cryochamber. The sections were then removed to room temperature and allowed to dry completely (30 min. - 1 hr.). Appropriate regions were trimmed from the tape-mounted tissues and attached, unfixed, to SEM specimen stubs. Gold-palladium sputter coated specimens were examined and photographed with an Eteck Autoscan electron microscope.

RESULTS

The processes of growth and maturation, represented in the disc-bone interface, create a more rigid union between these elements. The disc of the young individual is separated from the underlying vertebral cancellous bone by a relatively thick and discrete cartilage layer. Secondary centers of ossification associated with vascular invasion of this region, eventually form a continuous bony plate immediately below the annulus fibrosus,obliterating all but a thin remnant of the original cartilage structure. This process begins at the anterior-lateral margin of the inferior end-plate of each disc (Fig. 3). Fusion of discrete centers across the mid-line completes the process (Fig. 4).

While bony replacement of the cartilage plate has been initiated at the anterior margin, growth cartilage of the underlying cancellous bone is still active (Fig. 5) and many areas of contact between the end-plate and the underlying marrow spaces are retained (Fig. 6).

A feature, previously unreported, of this end-plate cartilage, is its mosaic appearance (Fig. 7). This is a reflection of denser territorial staining in comparison with the lighter staining interterritorial regions. This feature is present regardless of staining technique (Fig. 8).

Histochemical evaluation with Alcian blue demonstrates that the territorial regions are enriched with proteoglycans (Fig. 9). The interterritorial zone is conversely deficient in proteoglycan but does contain amorphous calcium salts (Fig. 10).

A similar picture emerges when this area is examined by scanning electron microscopy. A clear separation of territorial and interterritorial regions is visualized (Fig. 11). The textural elements of the fibrous and nonfibrous matrix elements are easily distinguished within these zones (Fig. 12). Cellular components are surrounded by a looser matrix than that of the more tightly packed interterritorial zone.

Fig. 1 View of microtome within LKB Cryochest. Specimen block is to left of knifeholder. Frontal sections are being cut. 1 6X (Total magnification given for all figures)

Fig. 2 Example of a reference photograph taken every 0.5 mm as specimen is sectioned. In this case the animal is identified as rhesus 495 and the section is 4.5 mm into the vertebral centrum in the sagital plane. 3/4X

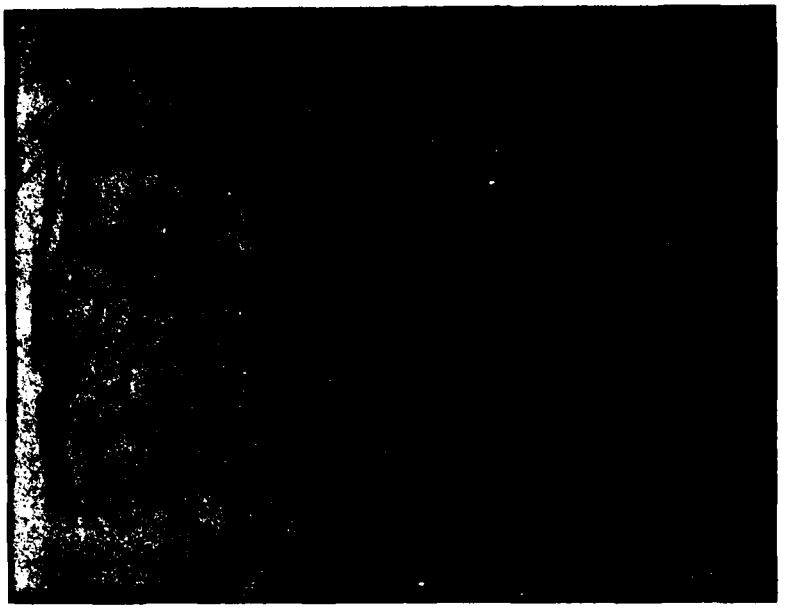

Fig. 3 Sagital section of rhesus T_2-T_3 Intervertebral Disc. Trabecular bone of vertebral body encloses marrow spaces. Cartilagenous end-plates are separated by growth cartilage from the adjacent spongy bone. Mallory, 7.5X

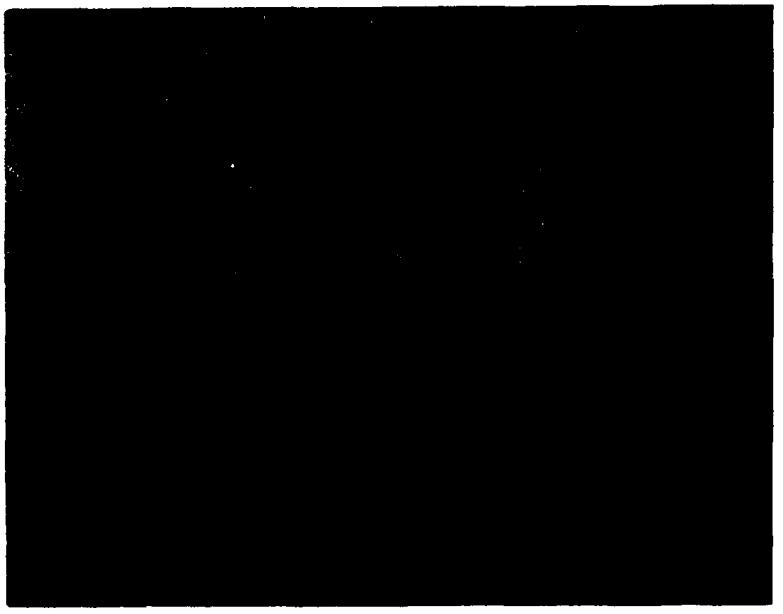

Fig. 4 Frontal Section of rhesus T_6-T_7 Disc. Vascularization of inferior end-plate is continuous but is restricted to lateral ends of superior. Mallory, 7.5X

Fig. 5 Low-power photomicrograph of anterior margin of inferior end-plate. Vascular buds are surrounded by ossifying matrix. Collagen bundles of annulus fibrosus above terminate in cartilage of end-plate. Light staining growth cartilage is located between end-plate and subchondral bone of vertebral centrum below. 50X

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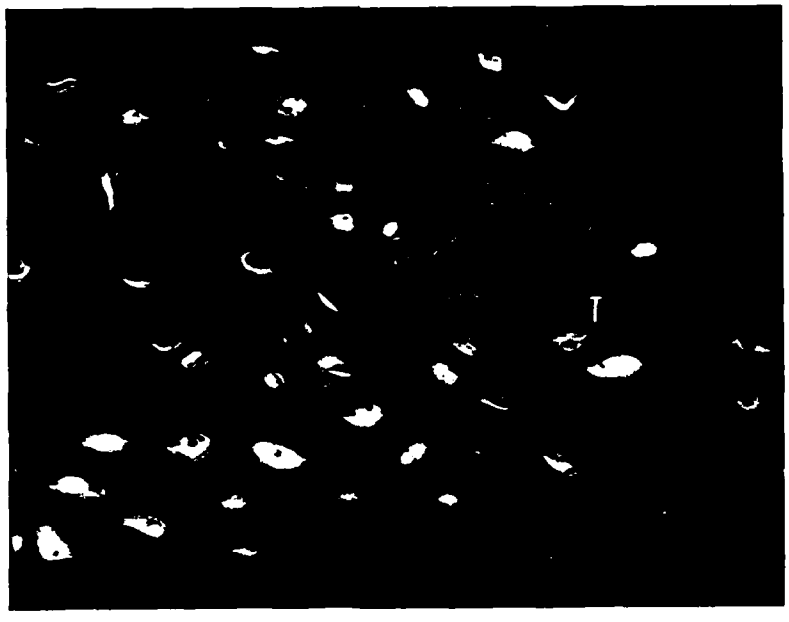

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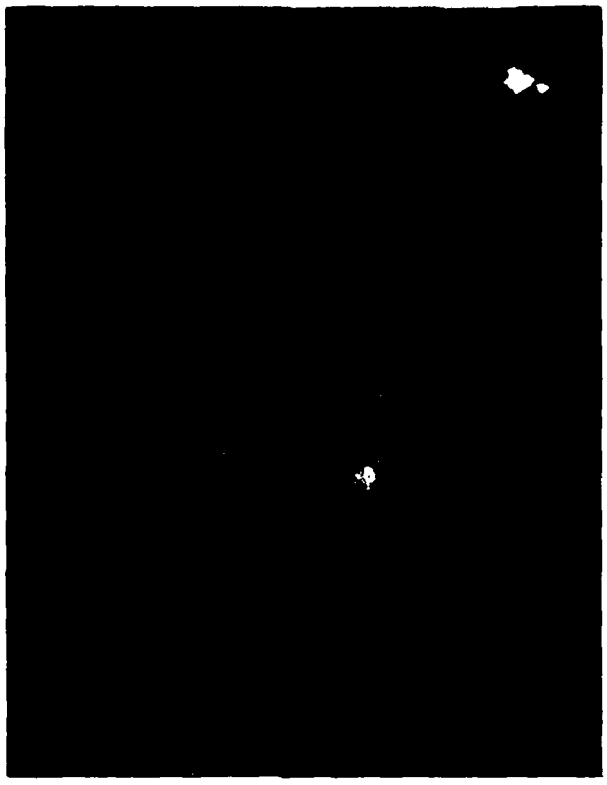

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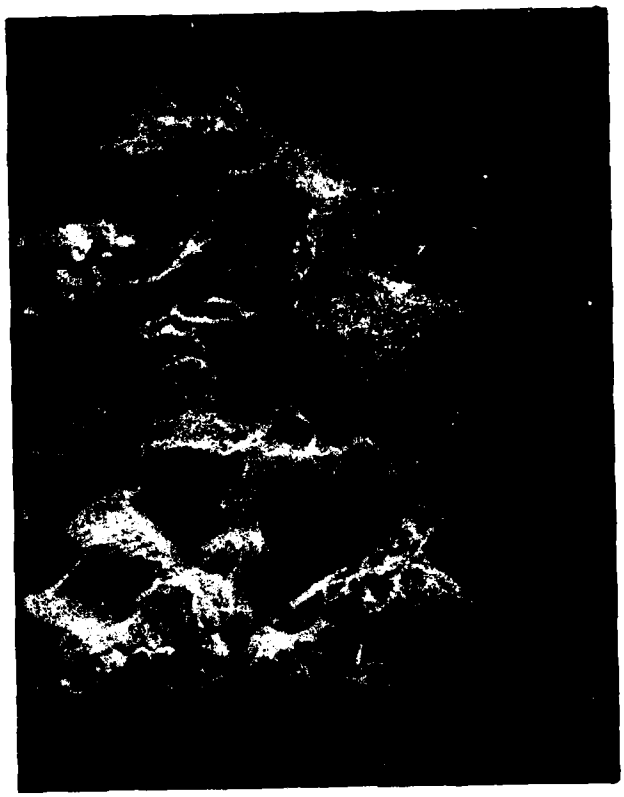

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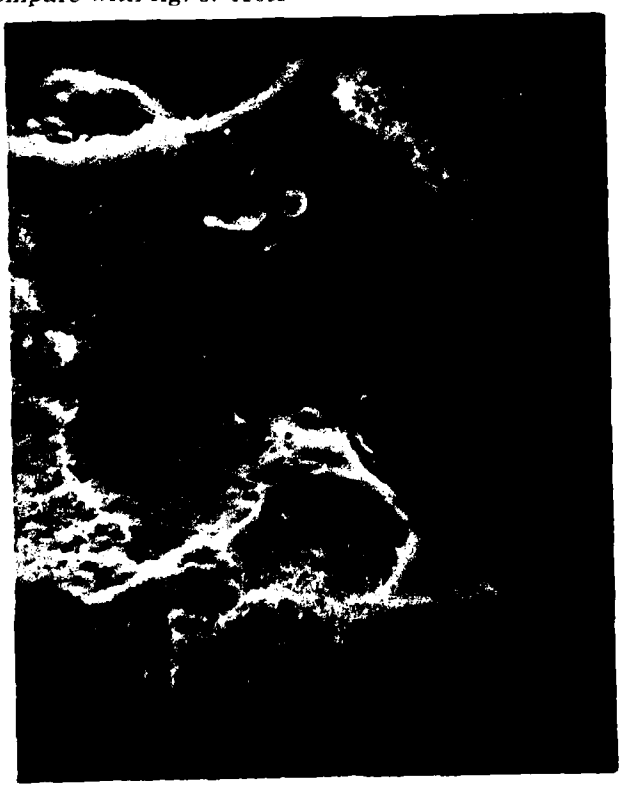

Fig. 12 SEM of cellular elements, territorial zone and adjacent calcified interterritorial matrix. 1400X

DISCUSSION

The intervertebral disc is specially adapted to resist compression loading while allowing spinal movement. It has been proposed that clinical disorders of the spine are the result of disc degeneration (Eyring, 1969). The mechanical properties of the disc are determined by the biochemical and biophysical state of the matrix components comprising the major substance of the nucleus pulposus and the noncollagenous elements of the annulus fibrosus (Muir, 1979; Lyons et al., 1981). This proteoglycan-enriched matrix is characterized by cell-mediated turnover, though the observed cell popolation is sparse (Nachemson et al., 1970).

After the age of 30 in humans, gradual changes in the biochemical and structural character of the discs become evident (Coventry, 1969). It has been suggested that these degenerative changes represent a form of accelerated aging (Harris & Macnab, 1954) and are responsible in part for the compromised biomechanics of the older spine. The nutritive needs of the cell populations within the nucleus and annulus compartments have been estimated (Maroudas et al., 1968) and it has been proposed that closure of the subchondral diffusion pathway, a consequence of ossification of the cartilaginous end-plate, is a determining factor in the ensuing cell death and matrix degeneration (Urban et al., 1977).

Histological examination of an aging series of human discs supported the significance of reduced trans end-plate diffusion in the etiology of disc degeneration (Nachemson et al., 1970). These and related studies consider the end-plates to be composed of hyaline cartilage and thus homologues of articular cartilage. Accordingly, diffusion and compression induced bulk flow are modeled upon data derived from examination of synovial joints (Brodin, 1955).

The results of this study in rhesus indicate, that while present, the cartilage of the end-plate is quite different in structure and presumably biomechanical function from that of articular cartilage. The identification of a heterogeneous extra-cellular matrix composed of calcified struts separating proteoglycan enriched territorial zones, suggests a unique relationship allowing for maximal linear strength coincident with prolonged cell viability. As ossification of this end-plate progresses, vascular invasion of the calcified matrix reestablishes a capillary bed immediately adjacent to the disc elements. Whether this vascular association is adequate to maintain cell function within the disc is dependent on structural and functional factors that are yet to be determined. Examination of undecalcified human disc is necessary in order to relate these observations to the clinical arena.

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